ATTORNEY DOCKET NO. 21085.0143U2
PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	
Anantharamaiah et al.	Group Art Unit: 1649
Application No. 10/712,447	Examiner: Daniel E. Kolker.
Filed: November 13, 2003	Confirmation No. 8707
For: SYNTHETIC SINGLE DOMAIN POLYPEPTIDES MIMICKING APOLIPOPROTEIN E AND METHODS OF USE	

DECLARATION OF GATTADAHALLI M. ANANTHARAMIAH UNDER 37 C.F.R. § 1.132

Commissioner for Patents
The Candler Building
Washington, D.C. 20231

NEEDLE & ROSENBERG, P.C.

Sir:

I, Gattadahalli M. Anantharamaiah, Ph.D., hereby declare that:

1. I am a co-inventor of the above-identified patent application.

2. I am a Professor in the Department of Medicine and the Department of Biochemistry and Molecular Genetics at the University of Alabama at Birmingham, and hold a Ph.D. degree from Bangalore University (India). I was appointed to the faculty of the University of Alabama at Birmingham as a Professor of Medicine in 1982 and served as Professor of Pathology until 1987 and continue to serve as a Professor of Medicine. In 1990 I was appointed the Senior Scientist at both the Center for Aging as well as the Center for AIDS Research at the University of Alabama at Birmingham and continue to serve in this role today. In 1992 I was appointed the Senior Scientist at the Comprehensive Cancer Center at the University of Alabama at

Birmingham and continue to serve in this role today. Additionally, in 1992 I was appointed to the faculty of the University of Alabama at Birmingham as a Professor of Biochemistry and Molecular Genetics. And in 1993 I was appointed Deputy Director of Administration of the Atherosclerosis Research Unit at the University of Alabama at Birmingham and continue to serve in this role today. I am the recipient of several awards including a million dollar National Institute of Health (NIH) grants for studies of Antiatherogenic Amphipathic Peptides: Structure & Function and Enhanced Hepatic Lipoprotein Uptake and Atherogenesis, both of which of which I am or was the project leader and/or the principal investigator.

3. Experiments conducted in my laboratory have demonstrated that a variety of apolipoprotein E mimicking peptides have the ability to decrease plasma cholesterol. Specifically, experiments conducted in my laboratory have demonstrated that a variety of apolipoprotein E mimicking peptides have the ability to enhance binding of low-density lipoprotein (LDL) or very low density lipoprotein (VLDL) to a cell. For example, the following sequences have all been shown to have such a function:

a. Ac-GIRRFLGSIWRFIRAFYG-NH_2 (the R-18L peptide) is a class L amphipathic helix with the lysine residues changed to arginine. Work in my lab as evidenced by the examples in the above-identified application provide evidence that this apolipoprotein E mimicking peptide has the ability to enhance binding of low-density lipoprotein (LDL) or very low density lipoprotein (VLDL) to a cell.

b. Ac-GIRRFYGSIWRFIRAFYG-NH_2 (the R-18L2Y peptide) is a class L amphipathic helix with the lysine residues changed to arginine and has the leucine residue at the sixth position of the peptide changes to a tyrosine residue. Work in my lab as evidenced by the examples in the above-identified application provide evidence that this apolipoprotein A mimicking peptide has the ability to enhance binding of low-density lipoprotein (LDL) or very low density lipoprotein (VLDL) to a cell.

4. Experiments conducted in my laboratory have demonstrated that truncated versions of the above apolipoprotein A mimicking peptides do not have the ability to decrease plasma

cholesterol. Specifically, experiments conducted in my laboratory have demonstrated that analogs of apolipoprotein E mimicking peptides do not have the ability to enhance binding of low-density lipoprotein (LDL) or very low density lipoprotein (VLDL) to a cell. For example, the following sequences have all been shown to be able to decrease plasma cholesterol:

a. Ac-(R)14L-NH_2 analog 1, having the sequence Ac-FLGSIWRFIRAFYG-NH_2, is a peptide derived from the original sequence (in this case R18L) and has the four C-terminal residues deleted. As such, this peptide does not fall within the scope of the pending claims. Specifically, two of the required Arg residues were removed in this peptide. Work in my lab as evidenced by the examples in the above-identified application provide evidence that this apolipoprotein E mimicking peptides does not have the ability to reduce plasma cholesterol.

b. However, Ac-(R)14L-NH_2 analog 2, having the sequence Ac-RRFLGSIWRFIRAF-NH_2, is a peptide derived from the original sequence (in this case R18L) and has two residues each deleted from the N-terminal and C-terminal ends of R18L, retains the critical Arg residues, and work in my lab as evidenced by the examples in the above-identified application provide evidence that this apolipoprotein E mimicking peptides has the ability to enhance LDL uptake and to reduce plasma cholesterol.

5. I along with others working in my laboratory, have also deduced and understood, based on molecular modeling experiments, that a lytic peptide can be made non-lytic by increasing the width of the polar face either by substituting Lys with Arg or by dimethylating Lys residues. This is supported by the results shown in Figure number two of the above-identified application and described in Example 2 of the above-identified application.

6. I along with others working in my laboratory, have further shown that a single amphipathic-helical structure containing Arg residues as well as a cluster of hydrophobic amino acids on the nonpolar face of the amphipathic helix is capable of associating with atherogenic LDL and VLDL and remnant lipoproteins to enhance their hepatic uptake and degradation, thereby reducing the overall level of plasma cholesterol. In other words, so long as the mimicking peptide comprises the sequence of X-Y-Arg-Arg-Y-Y-X-X-Y-Y-Arg-Y-Y-Arg, and

the only variability in the structure stems from the residues marked as "X" and "Y", and the peptide forms an amphipathic α-helical structure, then the amphipathic α-helical peptide will be able to associate with atherogenic LDL and VLDL and remnant lipoproteins to enhance their hepatic uptake and degradation, thereby reducing the overall level of plasma cholesterol We have also shown that the reverse is true, namely that if the alpha helical structure is disrupted by removing or replacing key amino acids, such as the Ac-(R)14L-NH_2 analog 1, the peptide will no longer be capable of reducing plasma cholesterol levels.

7. In summary, it has been demonstrated that a variety of different apolipoprotein E mimicking peptides are capable of decreasing plasma cholesterol levels, as most of the sequences given above. It has also been shown that the ability of the peptides to form an amphipathic α-helical structure by increasing the width of the polar face either by substituting Lys with Arg or by dimethylation of lysine will enhance binding of low-density lipoprotein (LDL) or very low density lipoprotein (VLDL) to a cell. Disruption of this structure removes the ability to decrease plasma cholesterol levels. In other words, based on my experience and the evidence discussed above, I conclude that the evidence indicates that so long as the peptides mimic apolipoprotein E peptides, namely by sharing a similar sequence and being able to form an amphipathic alpha helical structure with a widened polar face either by substituting Lys with Arg or by dimethylation of lysine, that the peptide will be able to decrease plasma cholesterol levels.

5. I declare that all statements made herein of my own knowledge and belief are true and that all statements made on information and belief are believed to be true, and further, that the statements are made with the knowledge that willful false statements are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 7/20/07

Gattadahalli M. Anantharamaiah, Ph.D.